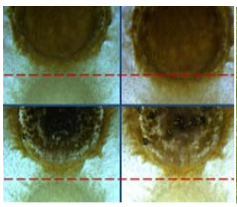
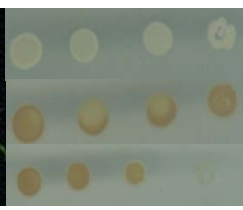
High School Students for Agricultural Science Research

Proceedings of the I Congress PIIISA – Estación Experimental del Zaidín





High School Students for Agricultural Science Research

Proceedings of the I Congress PIIISA program
(Science research by young students in Science, Technology and Humanities)

26th April 2012
Estación Experimental del Zaidín. CSIC.

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ISBN-10: 84-615-8735-9
ISBN-13: 978-84-615-8735-3

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PREFACIO

Lo que hay escrito a lo largo de estas páginas es el fruto de una andadura que comenzó en Noviembre de 2011: **el proyecto PIIISA2012**, al que algunos medios de comunicación calificaron como "Investigación con Acné". El nombre era de lo más sugerente y apropiado, pero había que darle forma y resultado. Han sido casi seis meses de esfuerzo con mayúsculas. Esfuerzo sobre todo por parte de los jóvenes (alumnos de secundaria y Bachillerato), pero también de los investigadores a su cargo, así como de los profesores coordinadores de secundaria.

Cada Proyecto ha tenido sus propias peculiaridades y dificultades, y como todo proyecto de Investigación, sus éxitos y sus fracasos; pero todos ellos han tenido un denominador común: la ilusión por conocer la ciencia y todos sus aspectos más escondidos. Los estudiantes han experimentado con la decepción, el acierto, la satisfacción final del trabajo acabado, la publicación y, lo más importante, la presentación a la sociedad que nos ha financiado y nos permite este lujo: el de INVESTIGAR.

Estos alumnos "privilegiados" han entrado en contacto con la CIENCIA en mayúsculas desarrollando experimentos nunca realizados por otros. A su vez los investigadores han tenido que ser lo suficientemente flexibles y dinámicos para combinar el objetivo de estos proyectos (ilusionar a adolescentes por la ciencia) con la realidad y prioridades de unos alumnos de secundaria y bachillerato (*el acné*). A este último aspecto los coordinadores del proyecto estaban mucho más acostumbrados.

El resultado lo podréis apreciar y disfrutar a lo largo de estas páginas, que seguro os sorprenderán. Que este libro sea no sólo un reflejo de lo que han sido estos seis meses sino el germen de un apasionante futuro.

Dr. Francisco Martínez-Abarca Pastor

PREFACE

What is written along these pages is the fruit of a long journey that began back in November 2011: ***project PIIISA2012****, which some media have described as "Research with Acne". The name was indeed very appropriate, but it was necessary to actually put it into shape. We spent almost six months to do that devoting much of our time and efforts, as did the teen students involved in the project and their teachers and persons in charge. Each Project has had its own peculiarities and difficulties, and as in every research project, its successes and its failures. Nonetheless, each and every one of them had a common denominator: the excitement of science itself and of all its more hidden aspects. The students have experienced disappointment, success and the final satisfaction of the work well done, as well as its publication and dissemination to the general public responsible of our financing and of making research real and tangible. These "privileged" students have come into contact with the SCIENCE, developing experiments that had never been done before. Researchers, on their side, had to be the sufficiently flexible and dynamic to combine the aim of these projects (making science exciting and attractive to teenage students) with their everyday life and top priorities (acne). Needless to say that the coordinators of the projects were far more familiarized with this unsightly problem than with the more scientific aspects.*

The results of this endeavour will be thoroughly enjoyed along these pages and will surely surprise you. We hope that this book will not only be a reflection of what these past six months have been, but that it will also set the basis for an exciting scientific future.

Dr. Francisco Martínez-Abarca Pastor

ÍNDICE

BIOTIC INTERACTIONS IN SOIL AND RHIZOSPHERE: PREDATION OF *SINORHIZOBIUM MELILOTI* BY *MYXOCOCCUS XANTHUS*

Gabriela Tarifa Álvarez[1,2], Ana Villar del Paso[1,2], Clara Pérez González[1,2], Mª Ángeles Yelamos Lorente[1,2], Irene Marín Sánchez[1,3], Nazaret Ceballos Miján[1,4], Raúl Peña López[1,5], Manuel Jurado de Haro[1,6], José Muñoz Dorado[7], Juana Pérez[7] and José I. Jiménez Zurdo[1*]

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SUMMARY

The nitrogen-fixing legume endosymbiont *Sinorhizobium meliloti* and the myxobacterium *Myxococcus xanthus* are both Gram-negative bacteria which could potentially interact in soil. The objectives of this research work were to assess predation patterns of *S. meliloti* by *M. xanthus* and to set up an experimental system to study this process. We first characterized the growth phenotypes of several *S. meliloti* strains and a reference *M. xanthus* strain in different culture media. We observed that the best medium for *S. meliloti* was the rich TY medium, where bacteria exhibited markedly exopolysaccharide (EPS)-dependent mucous and non-mucous phenotypes. In contrast, *M. xanthus* grew better in CTT medium where it showed a stable typical swarm phenotype. Co-inoculation assays on CTT plates showed two patterns of *S. meliloti* responses to predation by myxobacteria, likely dependent on EPS production. We next tagged *S. meliloti* strains by conjugation with a plasmid-borne kanamycin (Km) resistance gene. Predation assays in CTT broth revealed disappearance of *S. meliloti* tagged bacteria in the presence of *M. xanthus* as inferred from time-course plate counts in Km-supplemented TY medium. Our results provide a base-line to investigate the molecular bases of bacterial predation in the future.

INTRODUCTION

El suelo es el hábitat de una gran diversidad de microrganismos que interaccionan entre sí y con organismos superiores con los que conviven como las plantas. La rizosfera es una parte del suelo inmediata a las raíces donde se dan toda una serie de relaciones físicas y químicas que afectan a la estructura del suelo y a los organismos que viven en él. Además es el lugar de destino de carbohidratos y otros nutrientes que las plantas exudan por sus raíces para proveer de energía a los microorganismos, muchos de los cuales son beneficiosos y protegen a las plantas de organismos patógenos o contribuyen a su nutrición.

Este es el caso de la bacteria *S. meliloti*, la cual fija nitrógeno atmosférico ayudando a la planta después de haberse establecido endosimbióticamente dentro de nódulos radiculares de las leguminosas (*Fabaceae).* Esta bacteria no puede fijar nitrógeno atmosférico independientemente: requiere de una planta huésped que siempre es una leguminosa. Estas bacterias son Gram-negativas, mótiles y no esporulan. La fijación simbiótica del nitrógeno es

muy importante para la agricultura y la ecología pues hace que las plantas no necesiten del abono con fertilizantes químicos que causan daño al medioambiente.

Entre los habitantes del suelo también está *M. xanthus* que es una myxobacteria de vida libre (no establece simbiosis) de tipo bacilo muy alargado, con un ciclo repetido para su reproducción. Este ciclo se compone primero de la fase vegetativa que se da cuando tienen nutrientes en el medio, en donde comienzan a dividirse. Como son depredadoras, se alimentan de otras células vivas y necesitan actuar en grupo, en el caso de *M. xanthus,* en una especie de enjambre. Después de este ciclo comienza su ciclo de desarrollo donde la población se divide en distintas funciones y por último la germinación donde comienzan a reproducirse en forma de población.

La eficiencia de la simbiosis de *S. meliloti* con las plantas leguminosas depende entre otras cosas de la capacidad de las bacterias para sobrevivir en el suelo y la rizosfera. Por eso en este trabajo nos hemos planteado cómo responde *S. meliloti* al ataque de depredadores como *M. xanthus*.

MATERIALS AND METHODS

Bacteria and plasmids

Las bacterias y plásmidos utilizados en este trabajo se relacionan en la Tabla 1.

Tabla 1. Cepas bacterianas y plásmidos utilizados en este estudio

BACTERIAL STRAIN	**CHARACTERISTICS**	**REFERENCE**
Sinorhizobium meliloti 1021	Reference strain	Galibert *et al.*, 2001
S. meliloti AK21/27/28/70/83	Field isolates (Aral Sea)	Laboratory collection
S. meliloti RMO17	Field isolate	Villadas *et al*., 1995
S. meliloti GR4	Field isolate	Casadesús and Olivares, 1979
Escherichia coli DH5α	Laboratory strain	Bethesda Research Lab.
Myxococcus xanthus	Reference strain	Laboratory collection
PLASMID		
pSRKKm	pBBR1MCS-2 derivative, Km^r	Khan *et al.*, 2008
pRK2013	Helper plasmid for triparental mattings	Figurski and Helinski, 1979

Culture media

La composición de los medios utilizados para el cultivo de las cepas de *S. meliloti* y *M. xanthus* fue la siguiente:

CTT (para *M. xanthus*)

Bacto-Casitona	10 g/l
$MgSO_4$. 7 H_2O	2 g/l
Tampón fosfato potásico 0.1 M, pH 7,6	10 ml/l
Tampón Tris-HCl 1M, pH 7,6	10 ml/l
pH	7.6

TY (para *S. meliloti*)

$CaCl_2.2H_2O$	0,9 g/l
Triptona	5 g/l
Extracto de levadura	3 g/l

Los medios fueron esterilizados en autoclave (20 min a 120ºC) y cuando fue necesario se solidificaron con agar al 1,5%.

Conjugations

El plásmido con el gen de resistencia a Km (pSRK) fue movilizado a las distintas cepas de *S. meliloti* mediante cruces triparentales utilizando el plásmido pRK2013 como *helper*. Las cepas resistentes a Km (transconjugantes) fueron seleccionadas en medio TY suplementado con el antibiótico.

Predation assays

Las distintas cepas de *S. meliloti* se enfrentaron con *M. xanthus* en medio sólido y medio líquido CTT. En el primer caso las bacterias se cultivaron independientemente y después se enfrentaron en cada caso suspensiones con alta concentración de bacterias. Para los experimentos en medio líquido se co-inocularon matraces en medio CTT con *M. xanthus* y las depas GR4 y RMO17 de *S. meliloti* marcadas con el plásmido pSRK. La supervivencia de *S. meliloti* se determinó a distintos tiempos después de la inoculación de los matraces mediante conteo de colonias en placas de TY suplementado con Km.

RESULTS

Fenotipo de S. meliloti y M. xanthus en medio sólido

Para conocer el fenotipo de estas dos bacterias, las cultivamos independientemente en los medios TY y CTT. *S. meliloti* crece mejor en el medio TY y *M. xanthus* en el medio CTT. *S. meliloti* manifiesta mucosidad debido a la producción de un polisacárido extracelular. En cambio, *M. xanthus* crece en forma de enjambres en el medio CTT que aparecen en un tono amarillo. Nunca se formarán cuerpos fructíferos debido a que no se llega a agotar los nutrientes en el medio.

Patrones de resistencia de S. meliloti al ataque de M. xanthus

Enfrentamos *M. xanthus* con dos cepas de *S. meliloti* que tienen fenotipo diferente; mucoso (GR4) y no mucoso (AK21) (Fig. 1). Observamos dos patrones de respuesta al ataque de *M. xanthus*. Las bacterias no mucosas son muy susceptibles al ataque. Ya a las 48 h después de que conviven en la placa con *M. xanthus* se observa su lisis. A las 144 h todo el área de crecimiento de *S. meliloti* GR4 está completamente ocupada por bacterias de *M. xanthus* en su fase vegetativa. Por el contrario, las bacterias mucosas (*S. meliloti* AK21) son más resistentes al ataque de *M. xanthus*. No hay lisis aparente en las primeras 48 h y lo que se observa es que *M. xanthus* bordea a *S. meliloti* buscando un lugar donde penetrar. A las 172 h ya se observan signos de que *M. xanthus* ha provocado lisis de células de *S. meliloti* AK21.

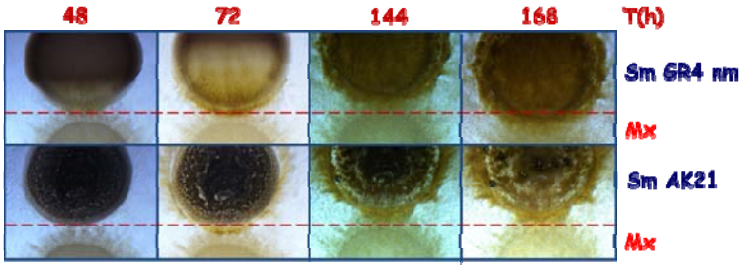

Fig. 1. Respuestas de *S. meliloti* (Sm) a la depredación por *M. xanthus* (Mx). El ensayo se realizó en medio sólido CTT con las cepas GR4 no mucosa (nm) y AK21 mucosa.

Ensayo en medio líquido para la investigación futura de la depredación bacteriana

En un sistema de medio sólido no es factible la investigación de las bases moleculares de este fenómeno, por eso ambas bacterias deben enfrentarse en experimento de depredación en medio líquido. Esto consistió en incubar *M. xanthus* con dos cepas de *S. meliloti* y en la valoración posterior de la supervivencia de *S. meliloti* a distintos tiempos. Para seleccionar las bacterias supervivientes antes del experimento marcamos *S. meliloti* con un gen de resistencia a Km (Fig. 2).

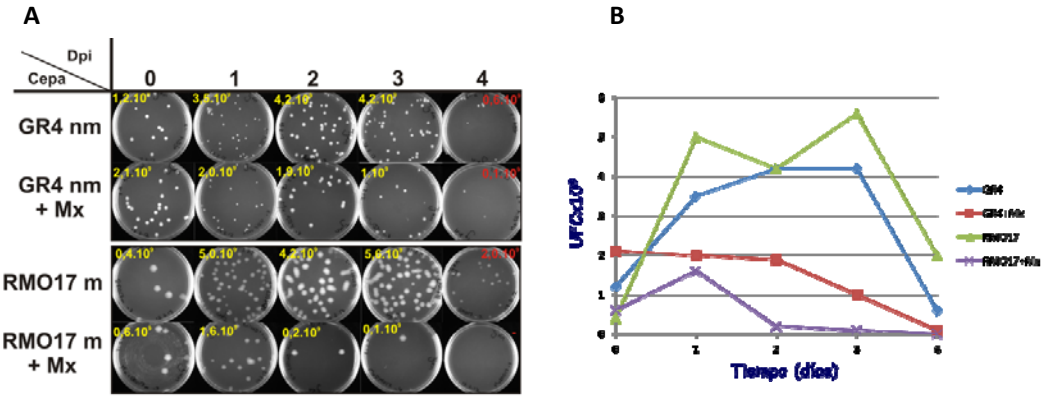

Fig. 2. Supervivencia de *S. meliloti* en medio líquido CTT en presencia de *M. xanthus*. A) Conteo en placa de colonias. Dpi, días post inoculación; B) Curvas de supervivencia. UFC, unidades formadoras de colonias. Mx, *M. xanthus.*

En la gráfica se muestran también las curvas de desaparición de *S. meliloti* cuando se cultiva en presencia y ausencia de *M. xanthus* en medio CTT. La cepa RMO17 tiene una supervivencia muy baja. En cambio, la cepa GR4 resiste más el ataque ya que al final del experimento todavía se puede contar alguna colonia.

CONCLUSIONS

1. *M. xanthus* is able to predate *S. meliloti* bacteria.
2. *S. meliloti* exhibits two different patterns of responses to predation, being the EPS-producing strains likely more resistant to the attack of myxobacteria.
3. A predation assay in liquid medium has been set up which will allow deciphering of the molecular bases of this process in the future.

ACKNOWLEDGEMENTS

This work was performed in the Microbiology and Symbiotic Systems Department at Estación Experimental del Zaidín (Consejo Superior de Investigaciones Científicas) within the frame of the Young Students in Science Program (PIIISA2012; http://emc2astronomy.blogspot.com/). It was mainly supported by research project MICINN Consolider-Ingenio 2010 CSD2009-00006 and Institutos de Educación Secundaria de Granada: Zaidín Vergeles, Angel Ganivet, Francisco Ayala, Mariana Pineda and Padre Manjón. We also appreciate the coordination of teachers Antonio Quesada, Francisco Javier López, Mª Concepción Martínez and Javier Cáceres. The technical support of Vicenta Millán and Ascensión Martos is also acknowledged.

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MY OWN IDEAS

Gabriela Tarifa Álvarez, IES-Zaidín Vergeles

This investigation has given me many ideas that I want to describe to continue this project. First of all, I'm going to talk about *Sinorhizobium meliloti*. The most important characteristic is that this bacterium is a nitrogen-fixing legume symbiont. *S. meliloti* produce nodules in the roots of host plants and help them to fix atmospheric nitrogen for growth. If plants have got nitrogen from bacteria they will not need any chemical fertilizer and it will be better for the soil and the environment. But, if that was impossible, why don't we try to use one fertilizer that doesn't inhibit the process of nitrogen fixation? Finally, I think it will be interesting to experiment the same process in other conditions. For example, what would happen if the humidity was higher in the environment?, or in freezing temperatures? Will this process continue without any problem?

The case of *Myxococcus xanthus*; this bacterium is a predator searching living cells to eat. My question is, why its preys don't offer any resistance by the time of depredation?, is there any other particular characteristic in *M. xanthus*?. This bacterium has got a social response by the time of hunt. Is this behavior codified by a gen?. If that is right, we could inhibit this and see what would happen. Furthermore, for this research, we know that *M. xanthus* predates *S. meliloti*. Could it exist another predator bacteria that could feed *S. meliloti*?. Is the exopolysaccharide (EPS) codified by a gen?. If so, we can isolate that gene in order to generate resistance in other bacteria.

Finally, we know that plants compete for the inorganic matter, thus I wonder if it was a relationship with *S. meliloti*. Are all competing for the atmospheric nitrogen?.

In all the investigation experience, I've learned too much about how scientist investigating and living for searching responses. In my future, I would like to be research scientist to learn more about the world of science.

Ana Villar del Paso, IES-Zaidín Vergeles

The knowledge of the rhizosphere is complex, at the same time interesting. We find further behaviors among bacteria that live there, such as the higher animals. In conducting this research, I realized that even the tiniest creatures, such as bacteria, need to guard against invaders that attack to take food to survive too. This relation that I have seen by watching *Mixococus xanthus*, depredate on *Sinorhizobium meliloti*, looking for food.

In order to address this phenomenon these two bacteria have had to grow various strains, especially *S. meliloti* (AK21/27/28/70/83, RMO17, GR4), we have seen grow best in what medium (CTT for *M. xantus* and TY for *S. meliloti*).

When *M. xantus* attacks, depending on the strain *S. meliloti*, it costs more or less work. At first try a frontal attack, if successful, start to lyse, if this fails, *M. xanthus* starts to surround and ends killing *S. meliloti*.

S. meliloti fixes atmospheric nitrogen through nodules, helping the legume *Medicago sativa* (alfalfa). Given all this, we could apply this research to help *S. meliloti* not to be attacked by any other bacteria in the proximity of alfalfa plants, so you can

determine their nitrogen nodules, including increasing the amount of *S. meliloti*, to turn these nodules and help raise even more to the plant.

Bacteria can replace chemicals that are used to improve crops, as these are very harmful to the environment, and bacteria are natural and not harmful at all.

Clara Pérez González, IES-Zaidín Vergeles

In this research we first need to know a bit of the bacteria. They are a large domain of prokaryotic microorganisms; there are million types of bacteria, many all of them necessary for the soil. We have learned that the bacteria are so fascinating and complex too. We have studied the resistance of different strains of *Sinorhizobium meliloti* (GR4 and RMO17 mucose and not mucose) to be predated by *Myxococcus xanthus*, because *M. xanthus* need to feed *S. meliloti* too.

I think all that we have done in this research can be applied to do several things. We could cultivate in a plant in the presence of *S. meliloti* for improve the growth of it. This can help the plant to receive more nitrogen (because *S. meliloti* provides the plant with nitrogen through the nodules), without having to use harmful products to the crops such as pesticides.

Mª Ángeles Yelamos Lorente, IES-Zaidín Vergeles

With this experience I have learned to reflect on the lives of so tiny and complex beings such as bacteria, although they seem so different from us, we have many things in common which are not appreciated till you are in contact with this topic, because you can have a slight idea, but it is more than that. As if they were people they defend themselves from predators, they use their resources to avoid to be eaten, that means they fight for their survival, as well as they search for food. They also have a way of life that in the case of bacteria which we have worked with, *Sinorhizobium meliloti* and *Myxococcus xanthus* is the soil. As I have mentioned before bacteria defend themselves against dangers and this work tried to see how *S. meliloti* reacted when it was preyed upon by *M. xantus*, as it is a predatory bacterium. Strains mucous offer greater resistance to be eaten' than non-mucous. But in the end all *S. meliloti* strains are preyed upon with more or less work.

And although the study is about bacteria, the rhizosphere plays an important role because it is the environment they inhabit. With this experiment we can contribute to a more sustainable ecology, using in this case, a bacterium *S. meliloti*. As it is harder to lyse mucous *S. meliloti* strains, the case would be this: growing plants in environments where only found *S. meliloti* mucous, then leads to more nodules in legumes, which contributes to capturing the nitrogen. This contributes to enhance crops without pesticides, which helps to avoid contamination.

Irene Marín Sánchez, IES Ángel Ganivet

This work has been conceived to get an idea of how scientists work, performing research and developing possible scenarios, which may be the solution to our problems.

Many questions raised throughout this research, some of them have been solved; while solving others new questions have emerged.

I have some clear ideas drawn from this project which are:

1. Bacteria are unicellular microorganisms that have a small footprint and exhibit different shapes.
2. Bacteria are the most abundant organisms on the planet and may form colonies.
3. *Myxococcus xanthus* is a myxobacterium presented in predatory self-organized colonies called swarms.
4. If any bacteria that will engulf *M. xanthus* are very strong, they have mechanisms in order to enter the barrier and to eat them.
5. *Sinorhizobium meliloti* is a bacterium which fixes atmospheric nitrogen in symbiosis with leguminous plants.
6. If we culture *S. meliloti* in a medium not specific to this type of bacteria they may not grow much or do not get to develop.

These basic ideas are the safest. But from these allegations I raised other questions. Some are:

1. Is it possible that other bacteria might prey on others that are predators as well?
2. In the case of *M. xanthus*, is it possible to be predated by other bacteria with another type of organization?

I think you may have a predatory *M. xanthus*, like all living things. I discovered that the world of bacteria is very exciting and very interesting, full of riddles and possible answers and are waiting to be discovered.

Raúl Peña López, IES Mariana Pineda

The objectives of this research work were to assess predation patterns of *S. meliloti* by *M. xanthus* and to set up an experimental system to study this process. In this project we have done this year, we learned a lot of things related to bacteria, their environment and division. All are things that we would not have known had without this wonderful project and great scientists who have taught us all this. It was a very interesting project where, if I could participate again without thinking twice.

I hope that our work has served to enable scientists to continue researching and knowing new things about *M xanthus*, *S. meliloti* and their way of living together, when one devours the other.

The project has been very interesting and educational but that we had applied more in my opinion, we should be more involved in doing experiments and culture media for bacteria breed, also could have tried to confront us in the lab, although that we had not achieved as it was a very delicate and complicated process, but it would have been interesting and educational.

My congratulations to those who have proposed this year the project and hope to continue doing so many years, it's great.

DETERMINATION OF INDIVIDUALIZED BREEDING METHODS OF *ANTHOCORIS NEMORALIS* NEONATE NYMPHS

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INTRODUCTION

Biological control is a method of controlling pests, diseases and weeds. It consists on using organisms in order to control populations of other organisms potentially dangerous when they increase their populations into levels where they can be considered as pests.

One of the important biological control agents for the control of arthropod pests is the *Anthocoris nemoralis* because it feeds on a variety of phytophagous such as aphids, mites and larvae of Diptera. This specie is very hardy and adaptable, able to live on various plants, both cultivated and spontaneous. An early introduction into an ecosystem could provide a high number of predators to attack harmful organisms.

Figure 1. Adult of *A. nemoralis.*

This treatment, being natural, is better than spraying chemicals, which are very effective in eliminating the noxious agent but also kill their natural enemies including the *A. nemoralis*.

The olive tree is one of the most important in Spain and especially in Andalusia, so their study is important to understand the interactions of the major insect pest of olives: *Euphyllura olivine* and *Prays oleae*. *A. nemoralis* could prey on these pests in their stage of nymph and adult. The release of *A. nemoralis* in the olive tree as a defense to these pests as well as strengthen the presence of natural populations of *A. nemoralis*, would be very useful as it could exert an important control on the populations of herbivores and thus avoid the use of chemicals products, harmful to the environment .

The study of development of *A. nemoralis* would be helpful to establish the ability of this insect in combating pests in its different development stages. However, there are no breeding methods that allow to identify neonates of this species and to track their development. This is why this study aims:

To determinate the individualized breeding method that minimizes the newborn children nymphs´mortality of *A. nemoralis*.

MATERIAL AND METHODS

Breeding Methods

The breeding started with commercially-obtained adult individuals of *Anthocoris nemoralis*. Those were extracted from the container and placed in a big box (19.8 x 24.4 x 28.6 cm) with green beans as a breeding support and as a hydration source. *Ephestia kuehniella* eggs were also added to ensure the correct feeding of the insects, as well as a paper towel to provide shelter and avoid the stress.

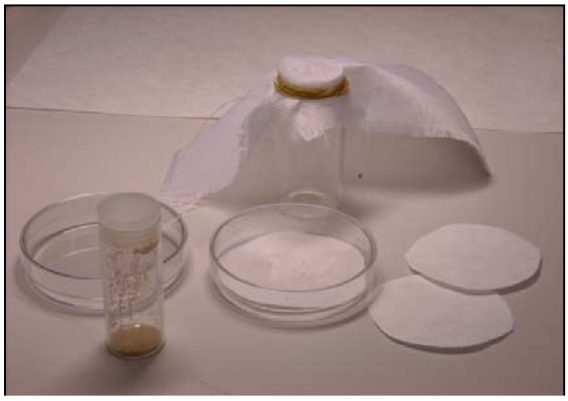


Figure 2.- Containers used in the experiment.

A few days later, the green beans were taken out and they were thoroughly examined on the magnifying glass to see how the eggs were "injected" in their skin. Some time had to pass until the eggs hatched and we could use the neonate nymphs in the proposed experiments.

Individualisation of neonate nymphs of *A. Nemoralis*

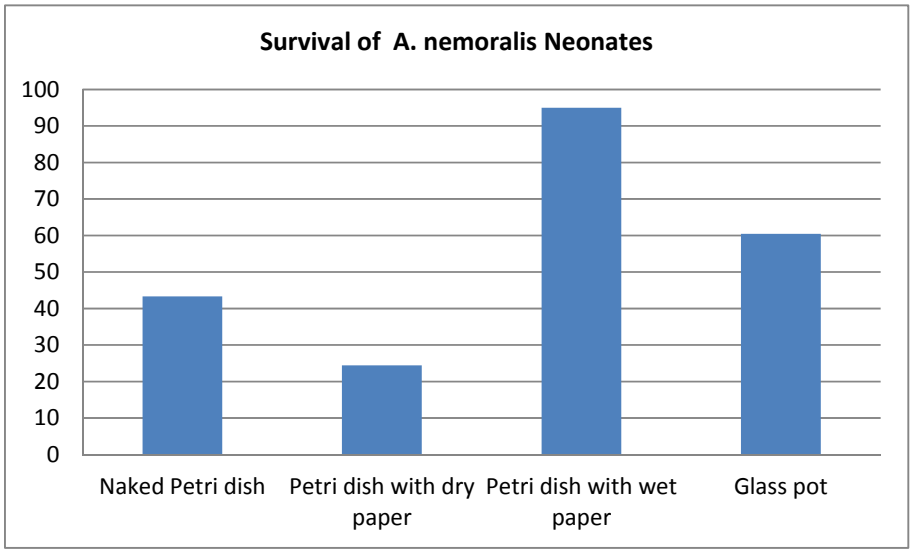
To determine the best way to raise from neonate nymphs of *A. nemoralis* on an individual container four possible methods were tested: naked Petri dish (diameter 5.7 cm and 1.5 cm height), Petri dish with dry paper, Petri dish with wet paper and glass pot (diameter 2.5 cm and 6 cm height) closed with a muslin tissue to allow ventilation inside the pot.

In blocks of ten individuals, three replicates of each treatment were done. In each of the containers neonate nymphs were introduced and a suitable amount of E. kuehniella eggs. After 24 hours individuals were observed to determine the status of the nymphs (alive or dead).

RESULTS AND DISCUSSION

In naked Petri dish 43,3% of neonates survived, in Petri dish with dry paper 24,4% survived. In

Petri dish with wet paper 95% and in glass pot the 60,4%.



The fact that the plate in which more individuals survive is the one that has humid paper it owes that the eggs of *E. khueniella* stick to the surface of the paper. This way, it is easier for *A. nemoralis*, whose mouth is a tubular appendix called rostrum, to introduce it on the egg and eat it. This is important, because newborn nymphs do not have enough strength to feed on the eggs if those are not hold.

CONCLUSION

We clearly observed that the best container for the neonates of *A. nemoralis* survival is the Petri dish with wet paper because it is where more nymphs survived.

MY OWN IDEAS

Nuria Sainz, IES Generalife

In this project I have learned a lot about many things: first of all, I have learned a lot of very interesting facts about *Anthochoris nemoralis*, an arthropod which I had never heard of it before. Secondly, I have got to know a little bit of how the scientific world works: from the labs to reading articles in magazines, even though I know that there are still a lot of things I have not experienced about this world yet. Finally, it has been a great project because I have met very good friends and we have done a great team work with such a good working atmosphere.
I hope this project continues and I would like to be enrolled in it next year for sure!

Elio Gugliere, IES Fray Luis de Granada

The project, overall, I think, would rather have done it on other complex known insect or a mammal, but this experience has helped me to see a little better how the world of biology and also the realization of a poster where we explained our project because it will last in the time.

El proyecto en general me ha gustado, hubiera preferido haberlo hecho sobre otro insecto más conocido y complejo o sino de algún mamífero, pero esta experiencia me ha ayudado a ver un poco mejor cómo es el mundo de la biología y también la realización de un póster donde explicamos nuestro proyecto porque eso perdurará en el tiempo.

DOES GARLIC-GENERATED NITRIC OXIDE (NO) AFFECT THE PHYSIOLOGY OF PEPPER FRUITS?

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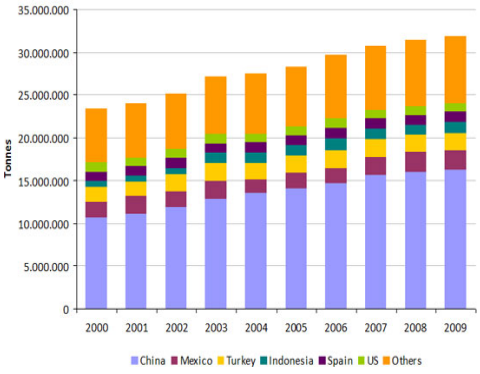
INTRODUCTION AND OBJECTIVE

The pepper is the fruit of a plant which belongs to the family of the Solanáceas. According to the shape, three main types of fruits are distinguished: California (square and short), Lamuyo (square and long), and dulce intaliano (long and narrow). Pepper varieties are classified as sweet and hot. Sweet peppers are usually red, yellow or green, with varying shapes and sizes. This group includes *morrón* and *dulce italiano*. Varieties from the hot peppers fruits are *Padrón*, *Gernika* and *piquillo*. The main pepper's producer countries are China, Mexico, Turkey, Indonesia, Spain and USA (Figure).



The NO is a colourless slightly soluble gas in water, which is present in small quantities in animals and plants. It is a very unstable molecule in the air, since it oxidizes rapidly in the presence of oxygen. In plants, NO intervenes in different physiological responses such as the opening and closing of the stomata and in the germination of seeds. Preliminary experiments indicate that garlic seems to be an important source of NO. In this project, we incubated pepper fruits with garlic and have studied how the fruits were affected.

MATERIALS AND METHODS

Plant materials

We have used pepper fruits (*Capsicum annuum* L., California type) supplied by Syngenta Seeds, Ltd. (El Ejido, Almería) and garlic purchased in a local market.

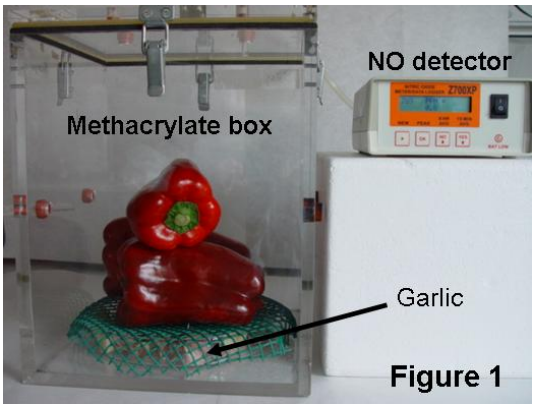
NO production by garlic

We peeled out the garlic and cut into different parts. Then, we placed the garlic in a flask connected to an NO detector. We estimated the ppm NO/g garlic.

Treatment of pepper fruits with garlic

We weighed each pepper individually. Then, we separated them into four independent groups: 1) Incubated with garlic for 1 hour into a methacrylate box (Fig. 1) and then at room

temperature (RT) for 3 weeks; 2) incubation without garlic and 3 weeks at RT; 3) Incubated with garlic for 1 hour and then at 5ºC for 3 weeks; and 4) incubated without garlic for 1 hour and 3 weeks at 5ºC.



Crude extracts

Pepper fruits were homogenized in a mortar in Tris-HCl 100 mM, pH 8.0, EDTA 1 mM, Triton X-100, 1% (v/v), glycerol 10% (v/v) and DTT 5 mM. Homogenates were centrifuged at 15.000 rpm for 10 min. Supernatants were used for assays.

Catalase activity

It was estimated by the method of Aebi (1984). Catalase can degrade hydrogen peroxide which can be measured by the decrease in the absorbance at 240 nm. Fifty µl of pepper extracts were added to 1 ml of a solution of K-phosphate buffer, pH 7.0 plus H_2O_2 10.6 mM. To prepare this solution 60 µl of hydrogen peroxide (30%) were diluted with K-phosphate buffer 50 mM, pH 7,0 to a final volume of 50 ml. Catalase activity was measured by the decrease of H_2O_2 at 240 nm during 2 min. at 25ºC in a spectrophotometer.

Protein determination

It was made following the colorimetric method of Bradford (1976). A standard curve was elaborated with different dilutions of bovine serum albumin (1 g/ml). Optical density of the solutions was measured at 595 nm in a spectrophotometer. Samples from pepper homogenates were compared with this standard curve in order to know their protein concentrations.

RESULTS

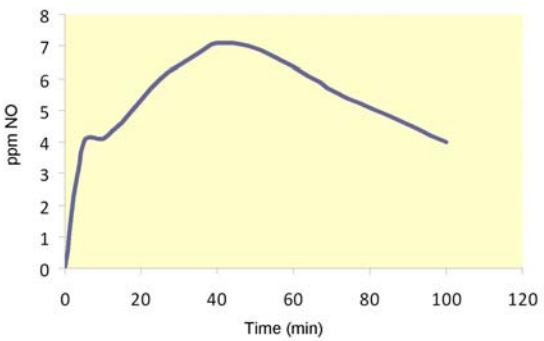


Fig. 2. NO production by garlic at different times.

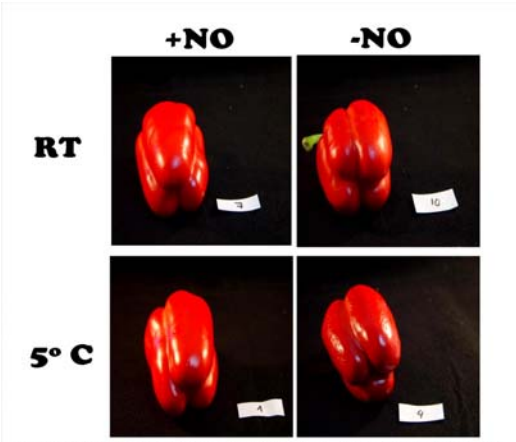


Fig. 3. Phenotype of fruits after treatment with (+) and without (-) garlic and further incubation for 3 weeks at room temperature (RT) and 5ºC.

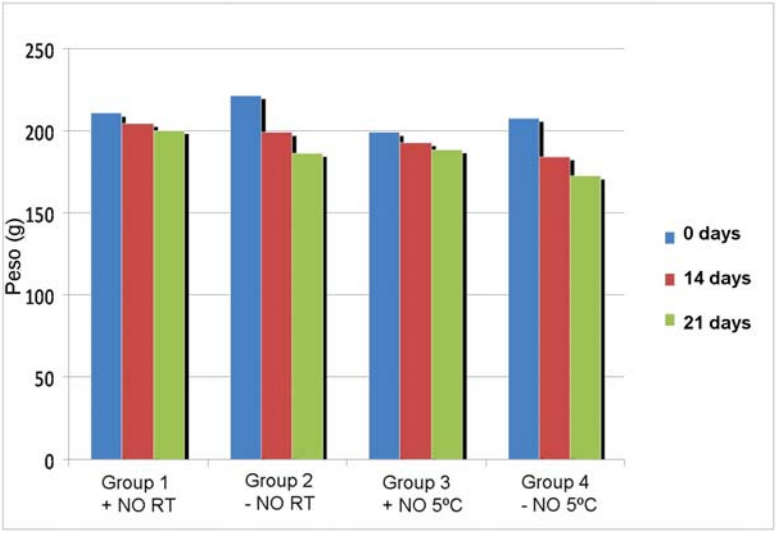


Fig. 4. Effect of incubation with garlic at different temperatures on the weight of pepper fruits after 3 weeks. RT, room temperature.

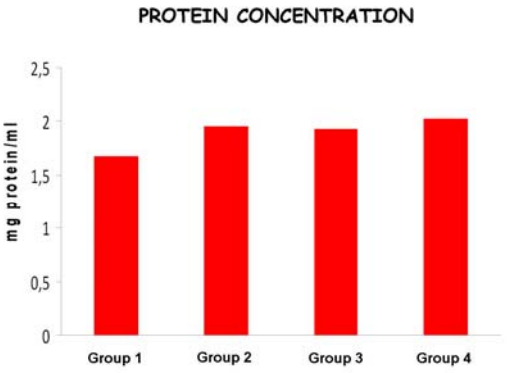


Fig. 5. Effect of incubation with garlic at different temperatures in the proteins concentration of pepper fruits.

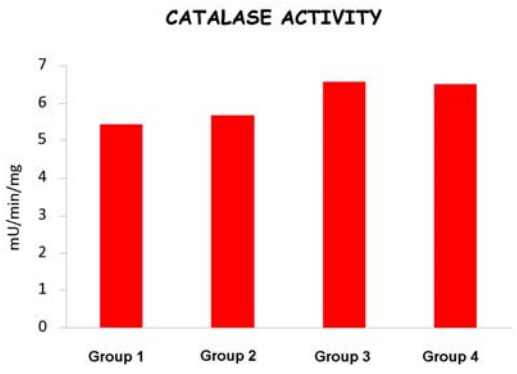


Fig. 6. Effect of incubation with garlic at different temperatures in the catalase activity of pepper fruits.

CONCLUSIONS

1. The incubation with garlic maintains the fresh weight of pepper fruits for weeks. Fruits not incubated with garlic lost about 20% of their fresh weight.
2. Storing fruits at low temperature (5ºC) affects the oxidative metabolism (catalase activity), but garlic treatment does not influence the response.

ACKNOWLEDGEMENTS

This work was supported by the ERDF-cofinanced grant AGL2008-00834 from the Ministry of Science and Innovation, Spain.

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MY OWN IDEAS

Nuria García Carbonero, IES Francisco Ayala

I think this project is very interesting because it allows us to get in touch with researchers and to learn more about this professional and everything related to it.
In addition, we performed real projects that have not been done before and we can obtain very important results with them. We have been introduced into the world of research and we have used materials and utensils that we have never seen before and that allowed us to learn lots of new things about this world. Researching with things of our everyday life, something as common as some peppers and garlic, you can know if their chemical interactions (in this case in the production of NO) can affect the maturation process and, therefore, to the physiology of these fruits. We have seen that with NO peppers lose less weight that peppers without NO at the same time. We also observed that keeping them in cold (5 °C), they are better preserved than at room temperature. I think that in general we have obtained good results, and they show that it is true that NO has effects on plants and it can influence them by altering their behavior, metabolism and physiology. It could be the basis for other researches later, and it would be a step forward because it would allow many of the fruit and vegetable processing companies to use this method to keep their products longer without using chemicals, and not only peppers, it could be extended to other types of fruits. I would continue investigating about this further to obtain all the possible conclusions I could and to get more benefits, and I would try to apply it to products that most interest us to facilitate their process of conservation and improve its quality, because we are the first to benefit from these advances.

Este proyecto me ha parecido muy interesante porque nos permite estar en contacto con investigadores y conocer más acerca de este ámbito profesional y todo lo relacionado con él.
Además, realizamos proyectos reales, que no se han hecho anteriormente y de los que se pueden obtener resultados muy importantes. Nos hemos introducido en el mundo de la investigación y hemos usado materiales y utensilios que nunca antes habíamos visto y que nos han permitido conocer infinidad de cosas nuevas sobre este mundo. Investigando con cosas de la vida cotidiana, algo tan común como unos pimientos y unos ajos, se puede saber si sus interacciones químicas (en este caso en la producción de NO) pueden afectar al proceso de maduración y, por tanto, a la fisiología de dichos frutos. Hemos podido comprobar que en presencia de NO los pimientos pierden menos peso en el mismo tiempo. También hemos observado que conservándolos en frío (a 5º C) se conservan mejor que a temperatura ambiente. Yo creo que en general hemos obtenido buenos resultados, y que indican que es cierto que el NO tiene efectos sobre las plantas y puede influir en ellas alterando su comportamiento, su metabolismo y su fisiología. Esto podría servir de base para otras investigaciones posteriores, y además sería un gran avance porque permitiría a muchas empresas del sector hortofrutícola utilizar este método para conservar sus productos durante más tiempo sin utilizar productos químicos, y no solo de pimientos, sino que posiblemente podría ampliarse a

otros tipos de frutos. Yo seguiría investigando sobre esto más a fondo para sacar todas las conclusiones posibles y conseguir muchos más beneficios, e intentar aplicarlo a los productos que más nos interesen para facilitar su proceso de conservación y mejorar su calidad, ya que nosotros somos los primeros en beneficiarnos de estos avances.

Carlos Nombela Durán, IES Padre Manjón

I think we have done an experiment wich can help us to discover too much about Nature and what we can do whit it. This experiment can be used in every house to help people to keep vegetables longer. This project has taught me something about a lot of laboratory instruments and how to use them. This experiment has permitted me to learn that most things that are in our homes, and can be used to do things that we don't know. In this time, I have seen a lot of chemicals, for example H_2O_2, that can be used to eliminate any imperfection in the skin as warts. I believe that we can't do this experiment whit other vegetable or other fruit, because the pepper has some enzymes and proteins that are unique on pepper fruits.

Pienso que hemos hecho un experimento que puede ayudarnos a descubrir muchas cosas acerca de la naturaleza y que podemos hacer con ella. Este experimento puede ser utilizado en todos los hogares para ayudar a las personas a mantener sus vegetales durante un tiempo más prolongado. Este proyecto me ha enseñado algunas cosas sobre los instrumentos de un laboratorio y cómo usarlos. Este experimento me ha permitido aprender que muchas cosas que hay en nuestros hogares pueden ser utilizadas para hacer cosas que no conocemos. En este tiempo, he visto muchos productos químicos, como por ejemplo H_2O_2, que podría ser utilizado para eliminar alguna imperfección de la piel como las verrugas. Creo que no podemos aplicar esos resultados a otros vegetales u otra fruta, porque los frutos de pimiento tienen ciertas enzimas y proteínas que son únicas en esos frutos.

María del Mar Quesada Díaz, IES Padre Manjón

PIIISA project (Project for iniciation to research and innovation in secundary education in Andalucia) is a project devoted to iniciate secondary school student to scientific research. With this activity the students get an inicial knowledge about the work in laboratories and they learn how a research project really is. In our case, we have investigated the role that the nitric oxide can have on pepper fruits. As a result of some experiments we have discovered that nitric oxide has a positive effect on peppers because it delays its ripening and it preserves the peppers in the best conditions of aspect and outer shape. On the other hand, nitric oxide does not have any negative effect in relation to composition and properties of peppers when it is added to them. We have confirmed it with the study of catalase activity because there is no differences in the activity between peppers treated with nitric oxide and other ones not treated

with it.
Garlic is a fruit that release a great amount of nitric oxide and the experiments have demonstrated that garlic may be very good for the conservation of the fruits. In my opinion this project can have a positive application in fruit trade because we have noticed that this molecule doesn't affect peppers and preserves them, on the contrary of other chemical products that are added to fruits and can damage them and cause health problems in human beings.
One advantage of using garlic as a source of nitric oxide is that this molecule have a natural origin. It is not obtained from chemical industry, besides this may be polluting.
In relation to the nitric oxide produce by garlic it would be interesting to study the effects of this molecule on other types of fruits. If the effect of garlic on ripening is confirmed and it is possible to preserve fruits without any damaging effect, this treatment could be economically important in future in relation to preservation and distribution of fruits in trades.
This experience has been very interesting for me because it has helped me to understand how a research project is and how to work in a research laboratory. My opinion about this project is very positive and I am very glad of having participate in it.

El proyecto PIIISA (Proyecto de Iniciación a la Investigación de Innovación de Secundaria en Andalucía) es un proyecto dedicado a la iniciación de alumnos de instituto en el ámbito de la investigación científica. Gracias a éste los alumnos adquieren un conocimiento mínimo acerca del trabajo en laboratorios y de lo que realmente supone llevar a cabo un proyecto de investigación. En nuestro caso, hemos llevado a cabo una investigación acerca del efecto que puede tener el óxido nítrico sobre los frutos de pimiento. Gracias a una serie de estudios que hemos realizado, hemos descubierto que el óxido nítrico tiene un efecto positivo sobre los pimientos, ya que retrasa su maduración y conserva los pimientos en condiciones óptimas de aspecto y forma externa. Por otro lado, el óxido nítrico no presenta ningún aspecto negativo en cuanto a la composición y propiedades del pimiento cuando se le añade a éste. Esto lo hemos podido comprobar mediante el estudio de la actividad de un enzima como la catalasa puesto que no hay diferencias en su actividad en los pimientos tratados con óxido nítrico y en otros que no están.

El ajo es un fruto que produce gran cantidad de óxido nítrico y con este se han realizado estudios con los que se ha descubierto que el ajo puede ser favorable para la conservación de los frutos. Creo que este proyecto puede tener una aplicación positiva en el ámbito comercial porque se ha descubierto que el óxido nítrico no afecta a los pimientos y los conserva, a diferencia de otros productos químicos que se añaden a otros frutos y que pueden causar anomalías y problemas de salud.

Una ventaja del uso de los ajos como fuente de óxido nítrico es que su origen es natural y no se obtiene por fabricación química que puede contaminar.

Un posible estudio que se podría hacer en relación a lo descubierto con el óxido nítrico producido por los ajos es estudiar qué efectos tiene esta molécula sobre otros tipos de frutos. Si realmente se comprobara que con el óxido nítrico se pueden conservar los frutos sin añadir ningún tipo de efecto perjudicial a los mismos, este tratamiento podría adquirir una gran importancia económica en un futuro en relación a

la conservación y distribución de frutos en el mercado.

Esta experiencia ha sido muy positiva para mí porque me ha ayudado a darme cuenta de cómo se lleva a cabo un proyecto de investigación, y de cómo se trabaja realmente en un lugar de experimentación. Mi opinión sobre este proyecto es muy positiva y me alegro de haberlo realizado.

DRY OLIVE RESIDUE BIOREMEDIATION BY NEW SAPROBE FUNGI

María Naranjo Márquez[1], Ignacio Romero García[2]**, Belén Sola Bullejos[2]**, José Manuel Ruiz Gonzalez[2]**, Alvaro Arregui Morales[2]**, Pablo Ortega Martín[2]**, Tomás Ortega Martín[2]**, Ramón de la Chica Mora[2]**, Carmen Fernández Lacal[1]**, David Jiménez Carretero[2]**, Elisabet Aranda[3], Inmaculada Sampedro[3], Inmaculada García-Romera[3*]

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***Los alumnos de este centro participaron en la ejecución del trabajo pero no en la fase final de elaboración de mis ideas*

SUMMARY

Dry olive residue (DOR), the by-product of the two-phase extraction process, is very rich in organic matter and nutritionally relevant cations. For this reason, the agronomic use of this waste has been suggested although DOR exhibits significant phytotoxicity due to its phenols content. We analyzed the impact of *Fomes sclerodermus*, *Fusarium graminearum* and *Epicoccum* sp in the detoxification of this waste. Inoculation of DOR with *F. sclerodermus* and *F. graminearum* did not affect its inhibitory effects. However, *Epicoccum* caused an effective phytotoxicity reduction of the residue. The decrease of phenol concentration and the high level of laccase activity cause by this fungus suggest that the phenolic compounds are one of the main factors implicated in the DOR phytotoxicity. Our result showed that the incubation of DOR with *Epicoccum* will open the way for the use of olive oil residues as organic amendment in agricultural soils.

ACKNOWLEDGEMENTS

This work was supported by the Ministerio de Economia y Competitividad (AGL2008-00572).

MY OWN IDEAS

María Naranjo Márquez, IES Francisco Ayala

The project was very interesting for me. Firstly we prepare the saprobe fungi that are going to bioremediate the olive mill dry residue. Then we cultivate these fungi with the residue for several weeks. And the most interesting part was to see how the plants can grown better in the residue incubated with the saprobe fungi than the residue without incubation that are phytotoxic.

I have enjoyed a lot doing this work, I have seen how is the life in a laboratory and how fantastic is to make life easy thought some experiments.

I think this project is useful because in Andalucia there are a lot of olive residues that produce a big pollution problem. It is a good solution to save the environmental.

FLOWER DEVELOPMENT AND POLLEN MORPHOLOGY IN *Cucurbitaceae*

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INTRODUCTION AND OBJECTIVE

Up to date, early flower development and the fundamental changes accompanying pre- and post-fertilization in *Cucurbitaceae* (watermelon, melon, zucchini, cucumber….) have not been sufficiently described at the morphological level. Some varieties of *Cucurbitaceae* recently emerged as commercial trade, are generating a great success because of their high quality (they are very sweet), and their great agronomic value (large size, resistance to diseases, high yield, harvesting time...). Production of these fruits in the greenhouse requires the plants to be pollinated effectively. Bees and bumblebees are introduced artificially in greenhouses and usually carry out this duty. However, the efficiency of the process is not very high at present and farmers need it to be improved. This requires detailed knowledge of how pollen production takes place, how and when the female flowers reach their receptivity, and whether pollen is viable and can or cannot germinate as well as many other details of fertilization.

The main aim of this work was to characterize the morphological stages of floral development in two plant models: watermelon (*Citrullus lanatus*) and zucchini (*Cucurbita pepo*), by analyzing some structural aspects of their flowers in detail.

MATERIALS AND METHODS

1. Flowers were obtained in the greenhouses of the University of Almería.
2. Photographs of flowers at different developmental stages were taken with a macro lens.
3. Conventional chemical fixation of anthers and gynoecia was performed, using 4% PF and 1% GA in 0.1M phosphate buffer pH 7.2, followed by dehydration in ethanol and infiltration by increasing the concentration of paraffin.
4. Several samples were processed identically, but infiltrated with Unicryl synthetic resin.
5. Semi-thin sections were obtained on a paraffin microtome, and ultra-thin sections were cut on an ultramicrotome.
6. Semi-thin sections were stained with methylene blue/toluidine blue mixture or with

PAS (staining of polysaccharides).

7. The microscopy techniques used were as follows:
   - Bright-field Light Microscopy (LM: Microscope Zeiss Axioplan),
   - Confocal Laser Scanning Microscopy (CLSM: C-1 Microscope Nikon), and
   - Transmission Electron Microscopy (TEM: JEOL Electron Microscope JEM1011) at 80 kV.

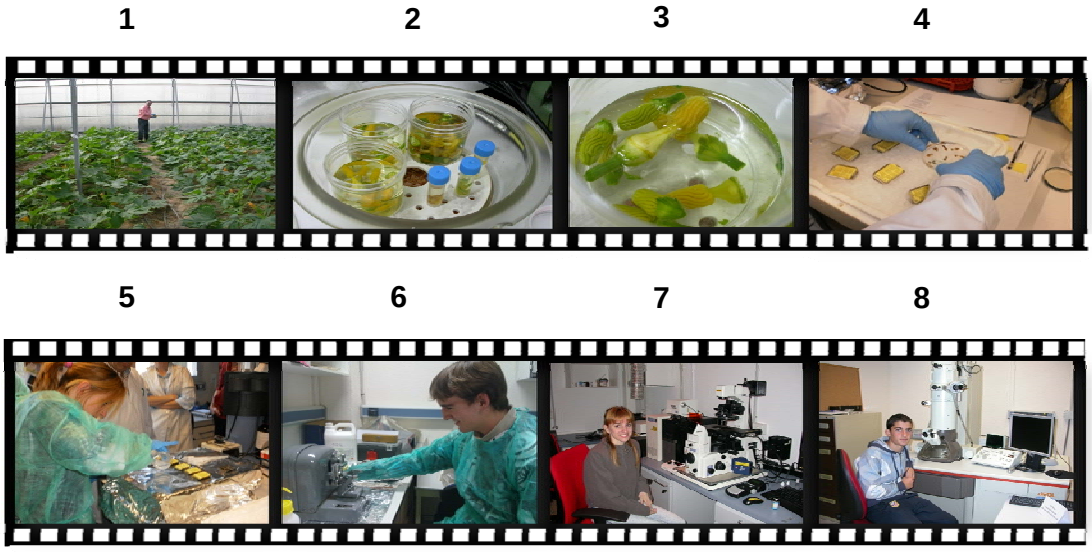

Figure 1. Photographic documentation of the key methodical steps of the study. (1) Collection of samples in UAL greenhouses, (2) Chemical fixation of the material, (3) Dehydration of the material (4) Infiltration of the material in paraffin and/or resin, (5) Polymerization of paraffin and/or resin, (6) Obtaining semi-thin sections, (7) Confocal microscope observations, (8) TEM observations.

RESULTS

1) FLORAL DEVELOPMENT IN ZUCCHINI

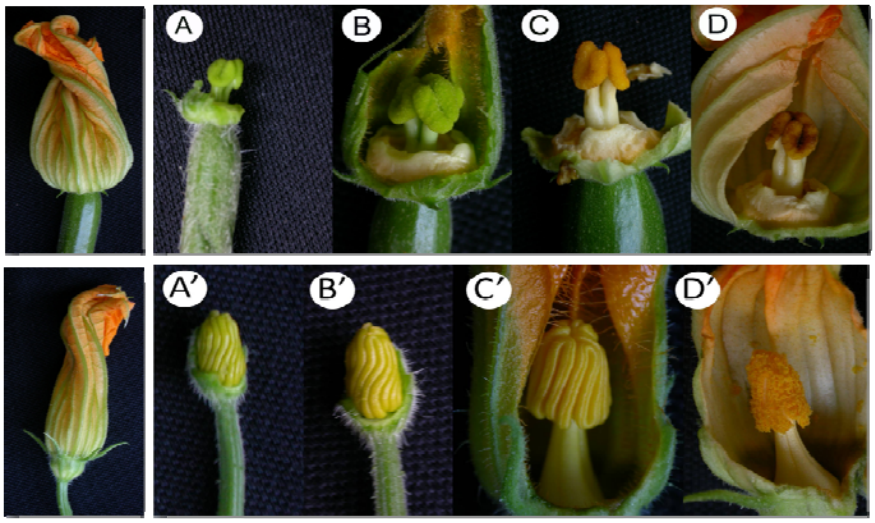

Figure 2. Floral development in zucchini. Top left: female zucchini flower. Upper row A-D: stages of zucchini pistil development. The pistil consists of three carpels fused in one ovary with 3 short styles partially fused at their base, each of which ends with a bilobed stigma. Bottom left: male flower of zucchini. Lower row, figures A'-D ': developmental stages of zucchini stamens. The male flower consists of 3 stamens, each one formed by a filament and a yellowish anther. The 3 stamens are fused at the filament and the anther (Figure 1C '). Pollen is orange-colored.

2) ANTHER DEVELOPMENT IN ZUCCHINI

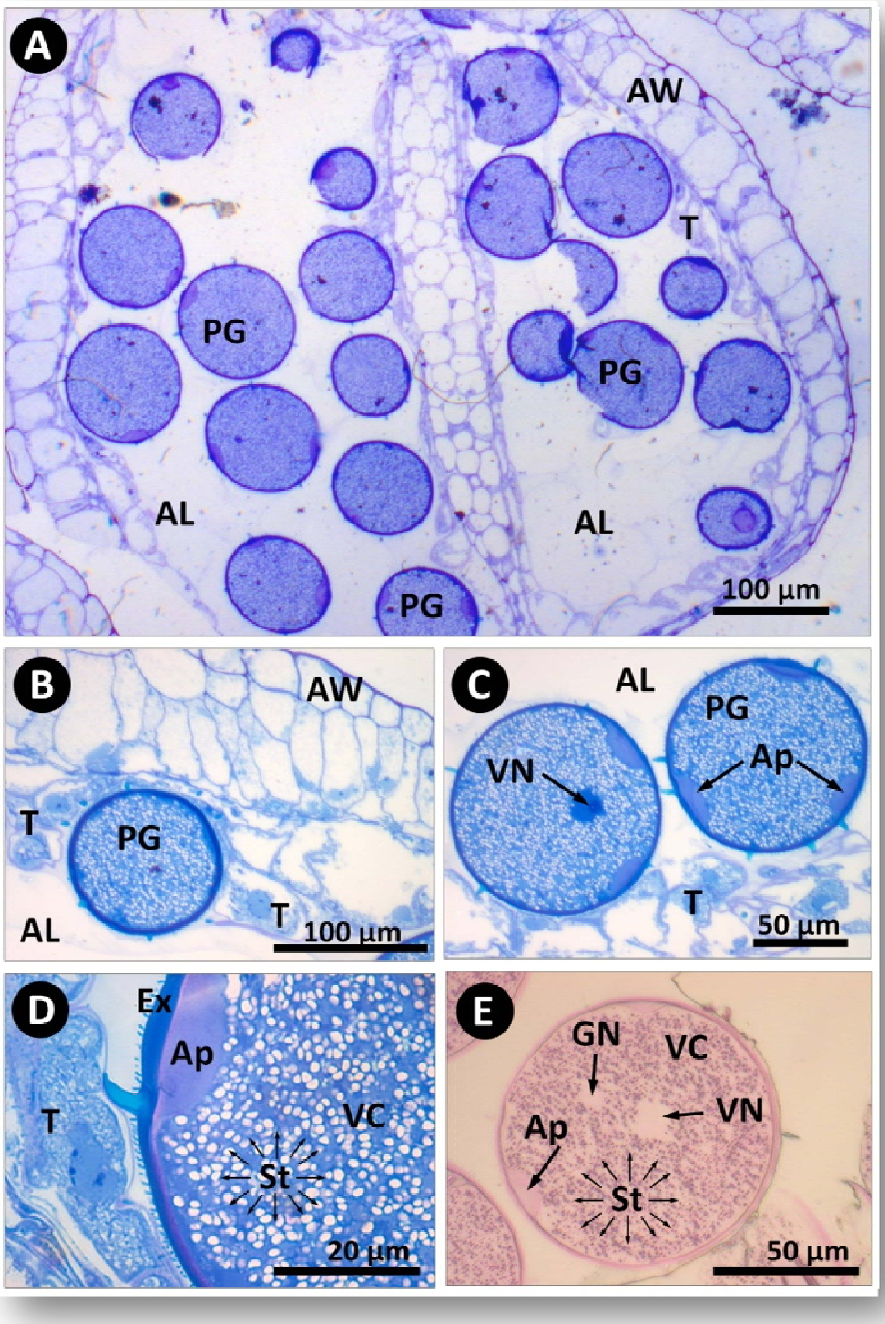

Figure 3. Sections of zucchini anther stained with methylene blue/toluidine blue mixture (B) or with PAS (E). Section of an anther showing numerous mature pollen grains in the locule (A). Details of various pollen grains located near the anther wall, surrounded by tapetum cells (B-C). Details of the vegetative cell cytoplasm and the apertural region, showing micro- and macro-spikes decorating the wall of the pollen grain (D). Numerous birefringent granules are observed in the cytoplasm, consisting of starch, as confirmed after specific polysaccharide staining with PAS (E). AL: anther locule, Ap aperture, AW: anther wall, Ex: exine, GN: generative nucleus, PG: pollen grain, St: starch, T: tapetum, VC: vegetative cell cytoplasm, VN: vegetative nucleus.

3) MORPHOLOGY OF THE ZUCHINI AND WATERMELON MATURE POLLENS

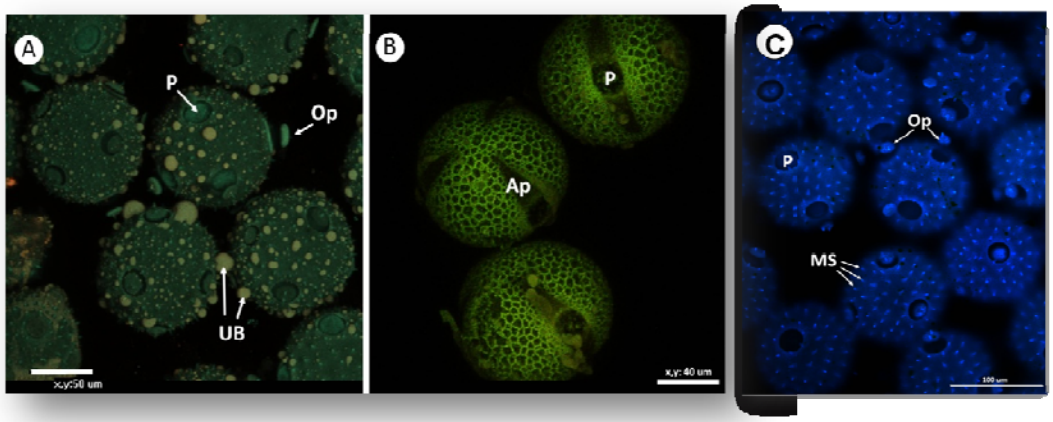

Figure 4. Mature pollen of zucchini and watermelon observed with a confocal laser scanning microscope (A and B) and with an epifluorescence microscope (C). (A) Projection of about 50 optical sections of zucchini pollen. The pollen is polypantoporated, nonpolar, with radial symmetry, circular and spherical. It possesses simple pore-type apertures and apertural membrane with operculum. Macro-spikes are present on the pollen surface. (B) Pollen from watermelon is trizoneporate, isopolar with radial symmetry. Simple apertures of the pore-type. (C) Autofluorescence of mature zucchini pollen excited with ultraviolet light. Pollen surface is of reticle-perforated type. Macro-spikes are especially well observed on the pollen surface. Op: operculum, MS: macro-spikes, P: pore. AP: aperture, UB: Ubish bodies.

4) ULTRASTRUCTURE OF ZUCCHINI MATURE POLLEN

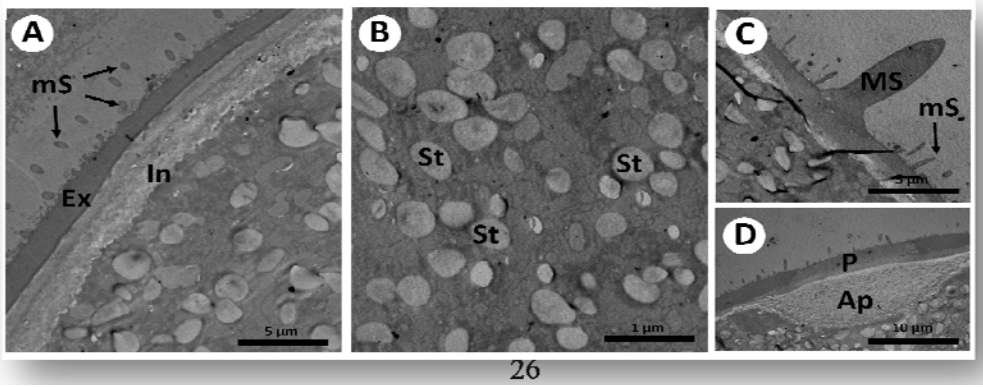

Figure 5. Mature pollen of zucchini observed with TEM. Sections were contrasted with uranyl acetate and lead citrate salts. Exine 4 µm of thick, with sexine approximately as thick as the nexine. Complete tectum, columelated infratectum. The pollen surface exhibits regularly distributed macro-spikes with a length of 5-9 µm, and numerous micro-spikes of 1 µm length. The pollen cytoplasm shows abundant starch granules. AP: aperture, Ex: exine, In: intine, mS: micro-spike, MS: macro-spike, Op: operculum, P: pore, St: starch.

CONCLUSIONS

1. The study of floral morphology in *Cucurbitaceae* is very important given the broad agronomic implications of this family and the frequent fertilization and fruit set problems present among their members.

2. The use of microscopy techniques with different levels of resolution allows us to observe details of the process and to connect them with the reproductive physiology of the plant.

3. Once the basic reproductive structure is known, it will be possible to analyze the behavior of commercial lines, hybrids, plants with different ploidy levels and so on, in order to try to improve some aspects of their reproduction, such as pollen loading, pollination efficiency and finally, their production.

ACKNOWLEDGEMENTS

This work was supported by FEDER funds: The project MEyC BFU2011-22779 and from "proyectos de excelencia": (JA) P2010-AGR6274, P2010-CVI5767 and P2011-CVI-7487. We would like also to thank C. Martínez Sierra for expert technical assistance, Prof. Mª Isabel Rodríguez-García for scientific discussion and the Confocal and Transmission Electron Microscopy (CTEM) core facilities of the EEZ-CSIC for providing excellent scientific environment.

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MY OWN IDEAS

This chapter compiles all ideas proposed by the high school students in round tables set throughout of the experiment.

- The experiments performed are enjoyable and interesting. We have worked with microscopes and other equipment we have never worked with or even seen before. On the whole, the experiments didn't seem difficult to be performed. The most difficult task of this study was paraffin embedding. What is the most difficult of these studies is how much time has to be used. They are quite aesthetic studies, as they are based in the capture of images from the microscopes, by observing flower elements.
- This kind of research on *Cucurbitaceae* has an agronomic value: by studying and using them in the products, resistance to virus diseases could be developed.
- By studying *Cucurbitaceae*, it could be found out which insects attack them, and achieve their resistance, avoiding their destruction by pests.
- The studies developed here could help to reach a higher (and more economically sustainable) yield, allowing better feeding of the population, which is considerably increasing yearly. Such higher production could be obtained by improving pollination efficiency in these plants.
- The analysis carried out could be used to produce new foods, medicines or elements helping society. A company could use this type of studies to create different and healthy foods, and also make money with these products.
- Another application of this study on the reproduction of Cucurbitaceae could be its use as a basis for further studies to be performed on similar species and families.
- Development of seeds could be also analyzed and nice and stunning images about their formation could also be obtained.
- I we know all these reproductive processes in detail, we could determine whether these plants can be mixed, and generate new hybrid fruits or modified plants of high interest.
- Another application could be to study pollen allergy in *Cucurbitaceae*, and how this may affect population.

HAS CUPPER ION ANY EFFECT ON *Sinorhizobium meliloti* BACTERIA?

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SUMMARY

Sinorhizobium meliloti is a soil bacterium that in symbiosis with Alfalfa leguminous plants fixes N2 to ammonia. This process occurs after an intimate communication between host and microsimbiont in the rhizosphere niche. This soil bacterium may suffer biotic and abiotic stresses in this complex environment. Resistance to any of this stresses can be an advantage for this type of bacteria. In this work we test the effects of Cupper ion in the bacterium *Sinorhizobium meliloti*. We found an effect on pigmentation and this effect is specific of each isolate (i.e. it is very pronounced for the isolate AK83 from Aral sea in Russia). On the other hand, comparison of Cupper ion resistance phenotype between isolates 1021 and GR4 suggests that the resistance of GR4 could be due to a set of 10 genes found in the cryptic plasmid pRmeGR4a of this strain. However, this resistance phenotype also appears in other isolates, and it could be explained by an alternative phenotype as Exopolysaccaride production (EPS+).

INTRODUCTION

Sinorhizobium meliloti es una bacteria del suelo que interacciona con plantas leguminosas de alfalfa estableciendo relaciones simbióticas donde la planta aporta el alimento y la bacteria fija el nitrógeno atmosférico (N2) transformándolo en amonio que la planta pueda aprovechar.

El establecimiento de la simbiosis es un proceso complejo dividido en varias fases. Tras un primer contacto y colonización de las raíces por parte del microsimbionte, se produce un intercambio de señales. Así, las raíces de las plantas liberan isoflavonas como señal de estímulo activando los factores de nodulación de estas bacterias. Estos factores de nodulación permiten la deformación del pelo radical (por donde se introduce la bacteria) e inducen paralelamente la organogénesis nodular. Es en el nódulo donde finalmente tiene lugar el proceso de reducción del N2 a amonio. La importancia del estudio de esta interacción radica en que este proceso simbiótico es el responsable de incorporar aproximadamente el 50% del Nitrógeno a los seres vivos.

Los pasos críticos de esta interacción tienen lugar en una zona de influencia de las raíces de la planta donde la actividad microbiana es muy intensa y que es conocida como rizosfera. Es en la rizosfera donde la bacteria se encuentra expuesta a numerosos estreses tanto bióticos (competencia por alimento,…) como abióticos (pesticidas, metales pesados,…).

Para enfrentarse a todo este conjunto de condiciones, *S. meliloti* posee un gran número de genes, siendo su tamaño genómico casi dos veces el de bacterias como *Escherichia coli*. Con las técnicas de secuenciación actuales, el conocimiento de todos los genes de una bacteria es una posibilidad real. De hecho empiezan a conocerse los genomas de distintos aislados de una misma especie. En el grupo de investigación donde se ha desarrollado este proyecto se acaba de obtener el genoma del aislado GR4 de la bacteria *S. meliloti* (Casadesus y Olivares 1979)

Como hecho destacado se han encontrado 10 genes presentes en el plásmido críptico pRmeGR4a relacionados con posibles transportadores, sensores y oxidasas del ion cobre.

El trabajo desarrollado ha tenido como objetivo estudiar la influencia del ion cobre sobre la bacteria *S. meliloti*. Para ello, se han abordado varios experimentos en medio sólido que muestran un efecto de este ion en la pigmentación de esta bacteria así como un fenotipo de resistencia variable entre los aislados.

MATERIALS AND METHODS

Bacterial strains

Table 1: Bacterial strains used in this work. ExoPolysaccaride producers (EPS+) and non producers (EPS-) are indicated

Bacterial strain	Relevant properties	Reference
Sinorhizobium meliloti 1021	Reference strain, EPS-	Galibert *et al*. 2001
S. meliloti GR4	Field isolate (Granada Spain), EPS-	Casadesus & Olivares 1979)
S. meliloti SM11	Field isolate (Germany) EPS-	Schneiker-Bekel *et al.* 2011
S. meliloti AK83	Field isolate (Aral sea, Russia) EPS+	Galardini *et al*. 2011
S. meliloti BL225C	Field isolate (Lodi, Italy) EPS+	Galardini *et al.* 2011

Drop assay

El ensayo de *drop assay* consiste en una medida semicuantitativa de crecimiento de bacterias en medio sólido (Figuras 1 y 2). Para su elaboración se trabaja en condiciones de esterilidad aprovechando, en nuestro caso, la campana de flujo laminar y el mechero Bunsen.

Preparación de placas

Se prepararon placas conteniendo medio sólido rico de crecimiento TY-Agar (20 ml). En nuestro experimentos, se añadieron además distintas cantidades de sulfato de cobre (desde un stock a 100 mM) que permitieron analizar curvas de concentración entre 0.0 y 1.2 mM de Cu. Se llevo especial cuidado en la homogeneidad del medio (utilizando el vortex y agitando las distintas soluciones).

Preparación de los cultivos.

Cultivos de la bacteria *S. meliloti* crecidos en medio liquido y en agitación durante 24-36h a 28^{o}C fueron cuantificados mediante medidas de turbidez en un espectrofotómetro a 600 nm de longitud de onda. Para ello se ajustó el cero de absorbancia con un tubo blanco conteniendo medio TY sin bacteria. Posteriormente, se miden los tubos conteniendo las bacterias y si el valor de Abs es superior a 0.8 ó 0.9 se diluyen ya que puede no ser fiable la medida. En ese caso se diluyen 5 veces (p. ej. 200 μl de bacteria en 800μl de TY). Esta medida, o sea, el valor obtenido de absorbancia para cada cultivo (fruto de multiplicar por cinco en el caso de que se hayan tenido que hacer diluciones para obtenerlo), es una medida indirecta del nº de bacterias presentes con las que hacer las diluciones seriadas.

Preparación de las diluciones seriadas.

Tras las mediciones anteriores, igualamos todos los cultivos hasta una OD de 0.5 a 600 nm. Cultivo al que llamaremos 10^0 y del que realizaremos las subsiguientes diluciones. Para ello, preparamos 4 tubos por cultivo que irán desde la dilución 10^{-1} hasta 10^{-4}. Cada tubo contiene 180 μl de Agua estéril. Una vez preparados procederemos a realizar las diluciones seriadas. Así, en el primer tubo (10^{-1}), añadiremos 20μl de bacteria procedentes del cultivo 10^0 y lo agitaremos en vórtex unos segundos para asegurarnos que la bacteria se ha diluido

homogéneamente. Para las siguientes diluciones se procederá de igual manera partiendo de la dilución anterior.

Depósito de las gotas

Cada una de las diluciones seriadas de cada aislado se dispondrá en una gradilla. Tras agitarlo de nuevo, 3 µl de cada una de las diluciones se pipetearon sobre cada una de las placas conteniendo distinta concentración de cobre; llevando cuidado de agitar de vez en cuando el stock de bacterias así como de utilizar una punta nueva en cada caso. Se procederá de menor a mayor concentración de bacteria.

Finalmente se permite que se sequen en la campana unos minutos antes de cerrar las placas e incubarlas a 30°C un mínimo de 3 días.

RESULTS

Para estudiar el efecto del ion cobre sobre *S. meliloti* se realizaron ensayos de "drop assay" enfrentando distintas diluciones de bacteria a distintas concentraciones de Cu2SO4. Dos tipos de experimentos fueron llevados a cabo dependiendo de la duración del ensayo. Así para los estudios de pigmentación, se trabajo en bajas concentraciones de cobre y tiempos de crecimiento largo, mientras que en los estudios de resistencia, se trabajó a mayores concentraciones y a tiempos de crecimiento más cortos.

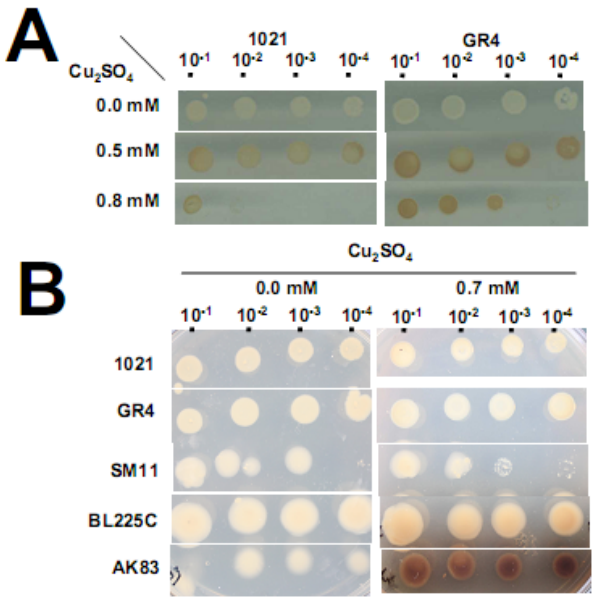

Figure 1: Low concentration of Cu (>0.8mM) shows an effect on *S. meliloti* pigmentation. Pictures show *S. meliloti* drop assays after 5 (B) or 8 (A) days of treatment. (A) Cupper effect on 1021 and GR4. Drop assay performed on different Cu2SO4 concentration (0.0, 0.5 and 0.8 mM) on TY plates using serial dilutions of TY liquid culture of GR4 and 1021 *S. meliloti* strains at 0.5 O.D. (B) Cupper effect on *S. meliloti* strains. Effect of 0.7 mM of Cu2SO4 on a Drop assay performed on TY plates using serial dilutions of TY liquid culture *S. meliloti* strains at 0.5 O.D. The strongest pigmentation is showed by AK83 strain.

Pigmentación

Tiempos largos de incubación frente al ion cobre permitieron apreciar un fenómeno de pigmentación de los distintos aislados de *S. meliloti* específicos de la presencia de este ion (Fig. 1). Esta pigmentación parece estar relacionada con la síntesis de melanina característica de algunos aislados de esta especie (Cubo *et al.* 1988). De hecho, la comparación de diferentes

estirpes permitió observar que en algunos aislados este fenómeno era mucho más acusado (Fig. 1B; p. ej AK83, aislado del mar de Aral en Rusia)

Resistencia

A diferencia del fenotipo de pigmentación observado tras largos periodos de incubación, el fenotipo de resistencia se analizó a tiempos más cortos (2-3 días) y a una curva de concentración del ion cobre de 0.0 a 1 mM (Fig. 2). La comparación entre el aislado de referencia 1021 y el que contenía el conjunto de genes relacionados con este metal (GR4) reveló que a partir de 0.6 mM se aprecia una diferencia en crecimiento entre ambos aislados, mientras que 1021 empieza a mostrar sensibilidad, GR4 no se ve afectada. El crecimiento de GR4 empieza a verse afectado a 0.9 mM de Cu (Fig. 2-A).

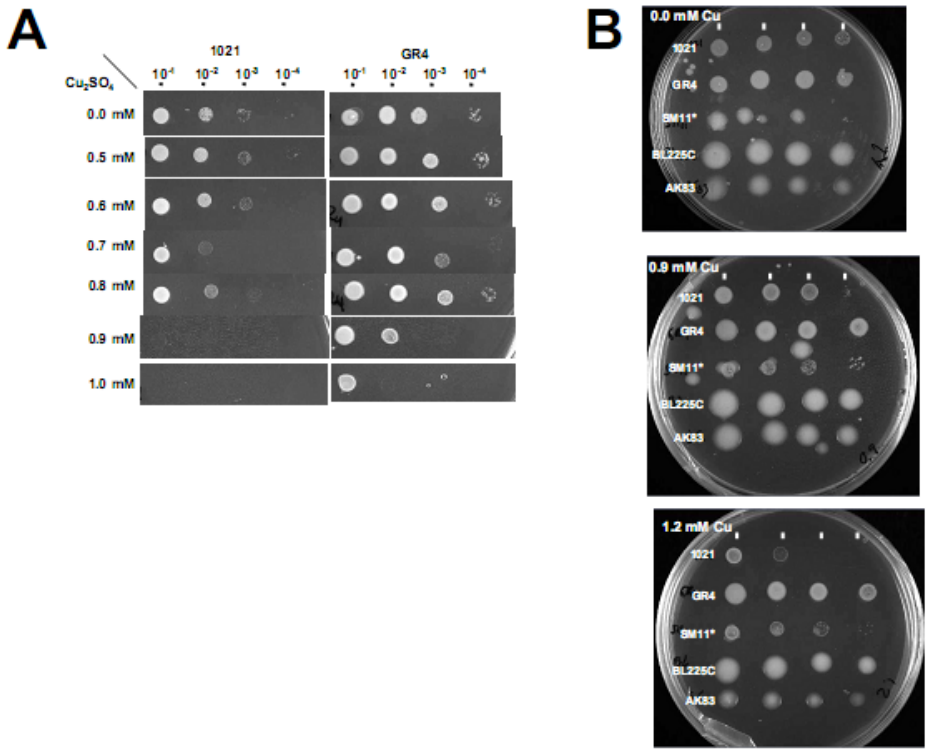

Figure 2: Cupper effect on *S. meliloti* growth

Pictures show *S. meliloti* drop assays after 72 h 30°C. (A) Cupper effect on 1021 and GR4. Drop assay performed on different Cu2SO4 concentration on TY plates using serial dilutions of TY liquid culture of GR4 and 1021 *S meliloti* strains at 0.5 O.D. GR4 shows more resistance than 1021 to Cupper ion.
(B) Cupper effect on *S. meliloti* strains. Drop assay plates containing different Cu2SO4 concentration (0.0, 0.9 and 1.2 mM) and *S. meliloti* strains (described on Table 1) after 72 h at 30°C. It must be noted that drops of SM11 strain (denoted with an asterisk) contain significantly less amount of bacteria.

Con objeto de determinar si este perfil de resistencia era una propiedad de GR4 ó bien un defecto de 1021, se analizaron nuevas estirpes de distinta procedencia (Tabla 1) y de las que se dispone de toda la información genética (Fig. 2-B). Como se puede apreciar, 1021, es la única estirpe que de forma clara mostró un fenotipo de crecimiento sensible al ion cobre. A la espera de conocer y comparar los distintos genomas de las bacterias analizadas sí podemos decir, que dos de ellas BL225c y AK83 presentan un fenotipo de exopolisacárido positivas (se incrementa el carácter mucoso en cultivo sólido) que puede tener un efecto generalizado en la resistencia a cobre frente a las cepas GR4 y SM11.

CONCLUSIONS

Low-concentrations of Cupper ion (<0.8mM) causes a pigmentation effect in the bacterium *Sinorhizobium meliloti*; this effect is specific of each isolate (i.e. it is very pronounced for the isolate AK83). Comparison of Cupper ion resistance phenotype between isolates 1021 and GR4 suggests that the resistance of GR4 could be due to a set of 10 genes found in the cryptic plasmid pRmeGR4a of this strain. However, this resistance phenotype also appears in other isolate, and it could be explained by an alternative phenotype as Exopolysaccaride producers (EPS+).

ACKNOWLEDGEMENTS

This work was performed in the Microbiology and Symbiotic Systems Department in the Estación Experimental del Zaidín – Consejo Superior de Investigaciones Científicas. It was supported mainly by research project MICINN Consolider-Ingenio 2010. CSD2009-00006; Scientific research by Young Students in Science (PIIISA2012; http://emc2astronomy.blogspot.com/). Institutos de Educación Secundaria de Granada: Padre Manjón, Angel Ganivet y Fray Luis de Granada. Also we appreciate the coordination of teachers Mª Concepción Martínez and Javier Cáceres.

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MY OWN IDEAS

Blanca Barón Torcal, IES Padre Manjón

Microbiology is the science of biology devoted to study the organisms that are only visible under a microscope: simple prokaryotes and eukaryotes. They are considered all living microorganisms. I think that microbiology is a science very important today because microbiologists have made contributions to biology and medicine, especially in the fields of Biochemistry, Genetics and Cell Biology. Microorganisms have many characteristics that make them "model organisms" ideal for study. Therefore, it is a very complex science with many aspects to consider for study.

The ecosystem *Sinorhizobium meliloti-alfalfa* has a very important impact in crops because it is a bacterium responsible for fixing nitrogen from the soil in leguminous plants. This feature has allowed us to advance the study of bacteria. Here we find the genome, (microbial genetics) that allows us to study the organization of microbial genes and how they affect different cells, which is very useful for Microbiology. Whenever we have a problem we solve first theory, then we put it into practice. In science it is the same, and like all sciences, microbiology is essential to corroborate the assumptions we make, and we do it through experience. First, we face a problem we want to solve; then, we developed a hypothesis to give a possible answer to this conflict. Then, we put this hypothesis into practice through experience, subjecting it to several cases, so we can determine the truth of such hypothesis. After the experience, we conclude developing a possible theory or law is met in all cases. This is the fundamental method used by science to solve any hypothesis. In the process one must be careful, meticulous, and use appropriate methods to reach the truth in as much detail as possible. So you have to devote much time to research and find an explanation for everything. When we know the results of scientific work is our duty to make them known to the community; either to help others and enrich work, or to simply expose people and data on any subject. Therefore, communicating data in a scientific project is the basis of any project, because if not we disclosed that work is useless, never come to light and consequently would not do advances in science research.

From my point of view, a good scientist should have some essential qualities, such as a tidy, organized, observing the order above all, it is also advisable to be careful, that is, note even the smallest fully in any investigation. But in my opinion, the most important thing is to be a person who is not tired of searching, research, know, know, have ambition and purpose of the work done to observe, learn and present the conclusions he draws, for enrich science and society of which it forms part.

La microbiología es la ciencia de la biología dedicada a estudiar los organismos que son sólo visibles a través del microscopio: organismos procariotas y eucariotas simples. Son considerados microbios todos los seres vivos microscópicos. Yo pienso que la microbiología es una ciencia muy importante en la actualidad porque los microbiólogos han hecho contribuciones a la biología y a la medicina, especialmente en los campos de la bioquímica, genética y biología celular. Los microorganismos tienen muchas características que los hacen "organismos modelo" ideales para su estudio. Por ello, es una ciencia muy compleja con numerosos aspectos a tener en cuenta para su estudio.

El ecosistema Sinorhizobium meliloti-alfalfa tiene una misión muy importante en los cultivos, ya que es una bacteria encargada de fijar el nitrógeno del suelo en las plantas leguminosas. Esta función nos ha permitido avanzar en el estudio de las bacterias. Aquí encontramos el estudio genómico, (genética microbiana) que nos permite estudiar la organización de los genes microbianos y cómo afectan a las distintas células, lo que resulta muy útil para la microbiología.

Siempre que tenemos un problema lo resolvemos primero en teoría, y luego lo ponemos en práctica. En la ciencia ocurre lo mismo, y como todas las ciencias, en la microbiología es fundamental corroborar las suposiciones que hacemos, y lo hacemos a través de la experiencia. Primero, nos enfrentamos a un problema que queremos resolver, a continuación elaboramos una hipótesis, para dar una posible respuesta a ese conflicto. Luego ponemos esa hipótesis en práctica a través de la experiencia, sometiéndola a varios casos, y así poder determinar la veracidad de esa hipótesis. Una vez realizada la experiencia, concluimos elaborando una posible teoría o ley si se cumple en todos los casos. Este es el método fundamental utilizado por la ciencia para resolver una hipótesis. En el proceso es imprescindible ser cuidadosos, meticulosos, y utilizar los métodos adecuados para alcanzar la verdad de la forma más detallada posible. Por ello hay que dedicar mucho tiempo a la investigación y buscar una explicación a todo. Cuando conocemos los resultados de un trabajo científico nuestra obligación es darlos a conocer, ya sea para ayudar a otros trabajos y enriquecerlos, o para, simplemente, exponerlos a la gente y aportar datos sobre cualquier tema. Por tanto, comunicar los datos de un proyecto científico es la base de ese proyecto, ya que si no se da a conocer no nos sirve de nada ese trabajo, nunca saldría a la luz y como consecuencia no haríamos avances en la ciencia de la investigación.

Desde mi punto de vista, un buen científico debe tener unas cualidades esenciales, como ser una persona ordenada, organizada, que respete el orden por encima de todo; también es conveniente que sea meticuloso, es decir, que tenga en cuenta hasta el más mínimo detalle en cualquier investigación. Pero en mi opinión, lo más importante es que sea una persona que no se canse de buscar, investigar, saber, conocer, con ambición y que tenga como finalidad de su trabajo el hecho de observar, conocer y exponer las conclusiones que saca, para enriquecer a la ciencia y a la sociedad de la que forma parte.

Ana Guardia Molina, IES Ángel Ganivet

This project allowed me to learn more about Microbiology. I believe the development of this discipline is very important because microorganisms have wide applications in the industrial field, in the production of pharmaceutical interest (like drugs) and biotechnology. Also play an essential role in research laboratories in Molecular Biology. I think, although the study of the genome of the bacteria is well advanced (in many cases is known entire genome of a bacteria species), there are a variety of genes not yet have information, so you should keep the focus on this area for development.

In this project I have learned how to construct a hypothesis and experimental design to resolve it. I have found that is quite difficult to solve a hypothesis because you do not know the final result of your experiments; sometimes they are unpredictable. In some cases the hypothesis may be wrong, in other cases, we can get to place completely different from what we planned our starting point.

It's important to communicate the results of scientific work because it is the only way that any society can meet and dispose of them if needed.

In summary, a good scientist must be calm, capable of reflection and discussion of his ideas, with a high level of English (and if is possible in other languages), and with the ability to properly expose his projects and organize a work-plan and ideas.

Este proyecto me ha permitido conocer más de cerca la microbiología. El desarrollo de esta disciplina me parece de gran importancia ya que los microorganismos tienen amplias aplicaciones en el terreno industrial, en la producción de interés farmacéutico (como los medicamentos) y biotecnológico. Además juegan un papel esencial en los laboratorios de investigación en Biología Molecular.

Opino que, aunque el estudio genómico de las bacterias está muy avanzado (puesto que se conoce en muchos casos todo el genoma de las mismas), hay gran variedad de genes de los que aún no se tiene información, por lo que se debería mantener la atención en este campo para su desarrollo.

En este proyecto he podido aprender cómo se construye una hipótesis y el diseño experimental para resolverla. He podido comprobar que es bastante difícil resolver una hipótesis ya que no conoces cómo acabaran los experimentos que realices. En algunos casos la hipótesis puede ser errónea, en otros, podemos llegar a un lugar totalmente distinto al que habíamos planificado en nuestro punto de partida.

Creo que es importante comunicar los resultados de un trabajo científico porque es la única forma de que toda la sociedad pueda conocerlos y disponer de ellos si los necesita.

Un buen científico debe ser una persona serena, con capacidad de reflexión y argumentación de sus ideas, con un alto nivel de inglés (y si es posible de otros idiomas), con la habilidad de exponer correctamente sus proyectos así como de organizar su trabajo e ideas.

Angela Liébana Medina, IES Fray Luis de Granada

In that science project, we have been studying *Sinorhizobium meliloti* behavior in different situations, all of them starred by cupper ion.

This project has let me see how a microbiologist works day by day, some of its techniques and methods, and how it can seem that he is working with anything, but however he is doing much more than anyone can imagine, because Microbiology is an important discipline that we can not underestimate, it works with microscopic forms of life but it is not a reason to look down on it because these microorganisms make fundamental processes as we can see. An example of it is the bacterium *S.meliloti*, which lives, forming nodules, in the roots of the *Alfalfa*, a kind of leguminous plant. A symbiotic relationship is been produced, because *S.meliloti* works as a nitrogen-fixing bacterium, a really important job for the correct

preservation of the environment. But in our project, we have studied other properties of the bacterium, which are related with cupper effects on it. It has been possible because of the knowledge of the genome of this specie, this fact, I think, is key on this kind of studies, because almost everything has something to do with genetics, just because genetic is the base of everything.

During this project I have being watching how a Microbiology laboratory works, how can go to waste everything if a little detail fails, and how can appear unexpected results, but nevertheless they are even more useful than any other that have been planned before. On the other hand I have realized that anything is so easy that it seems, results do not appear from nothing, each project takes its time to act and even more important its time to think.

In summary, I would like to say that it has been a grateful experience and above all I have learned about how my future may be.

Nuestro proyecto científico ha estado orientado al estudio del comportamiento de Sinorhizobium meliloti en diferentes situaciones, todas ellas protagonizadas por el ion cobre.

Este proyecto me ha permitido ver cómo trabaja un microbiólogo en su día a día, algunas de sus técnicas y métodos y cómo puede parecer que no está trabajando con nada, aunque sin embargo está haciendo mucho más de lo que cualquiera podría imaginar, porque la Microbiología es una importante disciplina que no podemos subestimar, trabaja con formas de vida microscópicas, pero no es una razón para despreciarla puesto que estos microorganismos son fundamentales en los procesos que sí que podemos ver. Un ejemplo de ésto es la bacteria S.meliloti, que vive formando nódulos en las raíces de la Alfalfa, una planta leguminosa. Se produce una relación simbiótica, porque S.meliloti actúa como una bacteria fijadora del nitrógeno, una tarea muy importante para la correcta preservación del medio. Pero en nuestro proyecto, hemos estudiado otras propiedades de la bacteria, que están relacionadas con los efectos que el cobre tiene sobre ella. Esto ha sido posible debido al conocimiento genómico de la especie, pienso que este hecho es la clave de este tipo de estudios, porque casi todo está relacionado con la genética, ya que la genética es la base de todo.

Durante este tiempo he visto cómo funciona un laboratorio de Microbiología, cómo puede desperdiciarse todo si un pequeño detalle falla, y cómo pueden aparecer resultados inesperados, que no obstante, pueden ser más útiles que ningún otro que hubiese sido planeado con anterioridad. Por otro lado, me he dado cuenta de que nada es tan fácil como parece, los resultados no aparecen de la nada, y cada proyecto lleva su tiempo de hacer cosas (actuar) y más importante aún su tiempo de pensar.

Para terminar, me gustaría decir que ha sido una experiencia muy agradecida, y sobre todo que he aprendido sobre cómo podría ser mi futuro.

Sara Miñán Polo, IES Padre Manjón

Microbiology is from my point of view a very interesting science because in science, in all the sciences, is necessary to know the basics, the fundaments. Microbiology is a basic science in biology. Thanks to their study, their understanding and communication can come to know the biology, so that science is essential. We can not forget that the field of microbiology is very large, as much or even more so than biology, a discovery leads to another, and this to one further, and so on, it is a never-ending story.

At the ecosystem *Sinorhizobium meliloti*-alfalfa is remarkable success in using commercial inoculants to improve crop yields of legumes as nitrogen-fixing bacteria. As a result of Rhizobium strains used are not only highly effective but are also very competitive with the indigenous populations of rhizobia. The genome of the bacteria is a very useful science, allowing us to progress in many areas of daily life, such as medicine, drugs, pharmacy, diseases, etc. Like all science, Microbiology must be corroborated by experiences that increase their accuracy and reduce any doubts. I think a very effective way to establish a theory or a simple deduction, the experience, the success is the key, that if he fails, the whole theory discovered and previously thought becomes false automatically. In the experimental work is needed great care with all the elements to enhance the chances of success of the experiment.

Once the experience and proven the veracity of the alleged theory, we develop a hypothesis and make known what we discovered. This is essential for the advancement of science, we must communicate the data from scientific papers to make them known, thus exposing a part of our discovery, we help other research, clarifying ideas and corroborate other, therefore see that it is absolutely essential. All hypotheses must be signed either by a research team, a student, teacher, or whoever. In my opinion it is important to know who or who have obtained such data and have given the light as if anonymous, anyone could appropriate the merit and establish the same hypothesis as his own.

Every good scientist should have a calm personality, because it takes a lot of patience in this area when doing research, laboratory experiences, all need to know everything to expect and take your time. One of the qualities required is a scientist, it is impossible that a disorderly person to carry out an investigation. Well, it's possible, but with many more difficulties. Physical disorders also lead to mental disorders; in consequence the research itself ends up being a huge mess.

A good scientist likes his job, likes to investigate, collect information and work hard, fail and never give up, try again, always be optimistic and applying for all their knowledge. In a research team, may members have different careers and therefore apply different inputs, making it much more efficient teamwork in these cases. A scientist must know how to work with other scientists, know to criticize and accept criticism on his own research. But, over all, enjoy what he does.

La microbiología es desde mi punto de vista una ciencia muy interesante, ya que en el ámbito científico, en todas las ciencias es necesario conocer las bases, los cimientos. La microbiología es la ciencia base de la biología, gracias a su estudio, su comprensión y su comunicación podemos llegar a conocer la biología; por tanto, esta ciencia es imprescindible. No podemos olvidar que, aunque es una ciencia ''base", el ámbito de la microbiología es muy extenso, tanto o incluso más que la biología; un descubrimiento nos lleva a otro, y éste a otro posterior, y así sucesivamente; es un nunca acabar. En cuanto al ecosistema Sinorhizobium meliloti-alfalfa es notable su éxito en la utilización de inoculantes comerciales para mejorar el rendimiento de las cosechas de leguminosas, ya que es una bacteria fijadora de nitrógeno. Como resultado las cepas de Rhizobium utilizadas no sólo son muy efectivas, sino que también son muy competitivas con respecto a las poblaciones autóctonas de rizobios.

El estudio genómico de las bacterias es una ciencia muy útil, ya que nos permite avanzar en muchos ámbitos de la vida cotidiana, como la medicina, medicamentos, farmacia, enfermedades, etc. Como toda ciencia, la microbiología debe de ser corroborada por experiencias que aumenten su veracidad y disminuyan las posibles dudas; me parece una forma muy eficaz para establecer una teoría o una simple deducción, la experiencia, el éxito de la misma es la clave, ya que si fracasa, toda la teoría descubierta y pensada anteriormente se vuelve falsa de manera automática. En el trabajo experimental es necesario un gran cuidado con todos los elementos para favorecer las posibilidades de éxito del experimento, una vez realizada la experiencia y comprobado la veracidad de la teoría supuesta, debemos elaborar una hipótesis y dar a conocer lo que hemos descubierto. Esto es imprescindible para el avance de la ciencia, debemos comunicar los datos obtenidos en los trabajos científicos para darlos a conocer, de esta forma, a parte de exponer nuestro descubrimiento, ayudamos a otras investigaciones, se aclaran ideas y se corroboran otras, por tanto vemos que es completamente esencial. Toda hipótesis debe ser firmada, ya sea por un equipo de investigación, un alumno, profesor, o quien sea, en mi opinión es importante saber quien o quienes han obtenido esos datos y los han dado a la luz, ya que si es anónimo, cualquiera podría apropiarse del mérito y establecer una misma hipótesis como suya.

Todo buen científico debe de tener una personalidad tranquila, ya que se necesita muchísima paciencia en este ámbito, a la hora de hacer investigaciones, las experiencias en laboratorio, todo requiere saber esperar y llevar todo a su tiempo. Una de las cualidades que debe tener un científico es el orden, es imposible que una persona desordenada pueda llevar a cabo una investigación, bueno, es posible, pero con muchas más dificultades, el desorden físico también lleva al desorden mental, por tanto la investigación en sí acaba siendo un desorden descomunal. A un buen científico le gusta su trabajo, le gusta investigar, recaudar información y trabajar horas y horas, fallar y no rendirse nunca, volver a intentarlo, siempre de forma optimista y aplicando para todo sus conocimientos; en un equipo de investigación, puede que los miembros tengan diferentes carreras y por tanto aplican diferentes aportaciones, por lo que es mucho más eficiente un trabajo en equipo en estos casos. Un científico debe saber trabajar con otros científicos, debe saber criticar y aceptar críticas, y sobre todo que disfrute con lo que hace.

Jose Manuel Sanjuán Parra, IES Padre Manjón

When I decided to become jumbled in this project I hoped that they gave me one of the projects that I selected; when they assigned to me this project I did not know which I was going to find.

In the scientific aspect, I have to review the importance of the microbiology in science since, although it seems insignificant, in the project I have realized that in these organisms occurs essential processes for the life, and like ecosystem, everything is absolutely important, the loss of some of them can suppose a disaster.

In this project about *Sinorhizobium meliloti* and the symbiosis formed with plants of alfalfa is observed an essential union for life. These bacteria fix nitrogen and they transform it into ammonia that the plants take advantage of, and it makes possible the existence and survival of the species, and that we use to make our molecules. In the study of microorganisms it is essential to know the genome, so that they give tracks us of its phenotypes and the possible consequence.

In order to solve hypothesis it is important to verify it and to demonstrate its veracity by means of an experimental design that is essential to make to obtain results that confirm or deny our hypothesis. Once obtained results its spreading is advisable, because it can even suppose an aid or the base for another project. In science we must work like a team, understanding by team not only when the members can see each other but when there is a flow of information independently of the place where their members are.

For this type of work, the certainty the tenacity, the patience, the good one for making, for letting to a side the individualities to work as team is necessary personal values at the time of working.

In conclusion, although not project was one of my first options I am glad to have been in this, because a world related to the investigation has been opened in front of me, that it attracts to me, but puts difficult still more the election of a future profession.

Cuando decidí involucrarme en este proyecto esperaba que me dieran uno de los proyectos que yo preseleccioné; cuando me asignaron este proyecto no sabía con lo que me iba a encontrar.

En el aspecto científico he de reseñar la importancia de la microbiología en la ciencia ya que, aunque parece insignificante, en el proyecto me he dado cuenta que en estos organismos se dan procesos necesarios para la vida, y que como ecosistema, todo absolutamente es importante, la pérdida de alguno de ellos puede suponer un desastre.

En este proyecto acerca de Sinorhizobium meliloti y la simbiosis formada con plantas leguminosas de alfalfa se observa una unión esencial para nuestra vida. Estas bacterias fijan el nitrógeno atmosférico y lo transforman en amonio que aprovechan las plantas.

En el estudio de microorganismos es esencial conocer el genoma, de manera que nos den pistas de sus fenotipos y de las posibles consecuencias de los mismos.

Para resolver hipótesis es necesario en primer lugar, comprobarla y demostrar su veracidad por medio de un diseño experimental, que es imprescindible realizar para obtener resultados que confirmen o desmientan nuestra hipótesis de partida. Una vez obtenidos resultados es conveniente su divulgación, porque puede suponer una ayuda o incluso la base para otro proyecto.

En ciencia debemos trabajar como equipo, entendiendo por equipo no solo cuando se trabaja codo a codo sino cuando hay un flujo de información independientemente del lugar donde se encuentren sus miembros. No existen informaciones individuales, ya que la aportación de cada uno de los miembros de este equipo, es útil para otros miembros y otros equipos. Para este tipo de trabajo, la constancia la tenacidad, la paciencia, dejar a un lado las individualidades para trabajar como equipo son valores personales necesarios a la hora de trabajar.

En conclusión, a pesar de que no era una de mis primeras opciones estoy contento de haber estado en este proyecto porque se ha abierto ante mí un mundo relacionado con la investigación, que me atrae, pero pone aún más difícil la elección de una futura profesión.